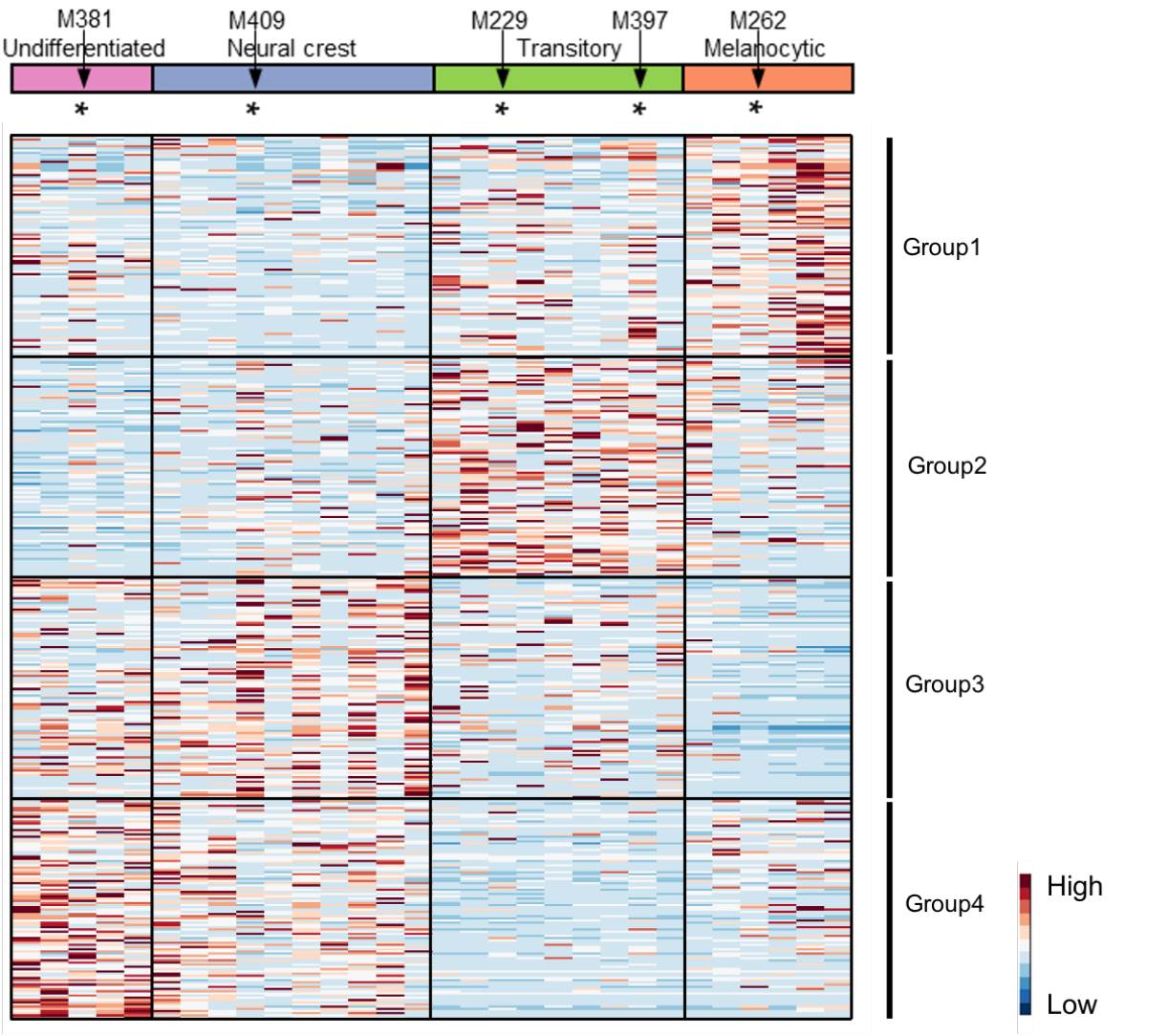
Supplementary Information

Raman-guided Subcellular Pharmaco-Metabolomics for Metastatic Melanoma Cells

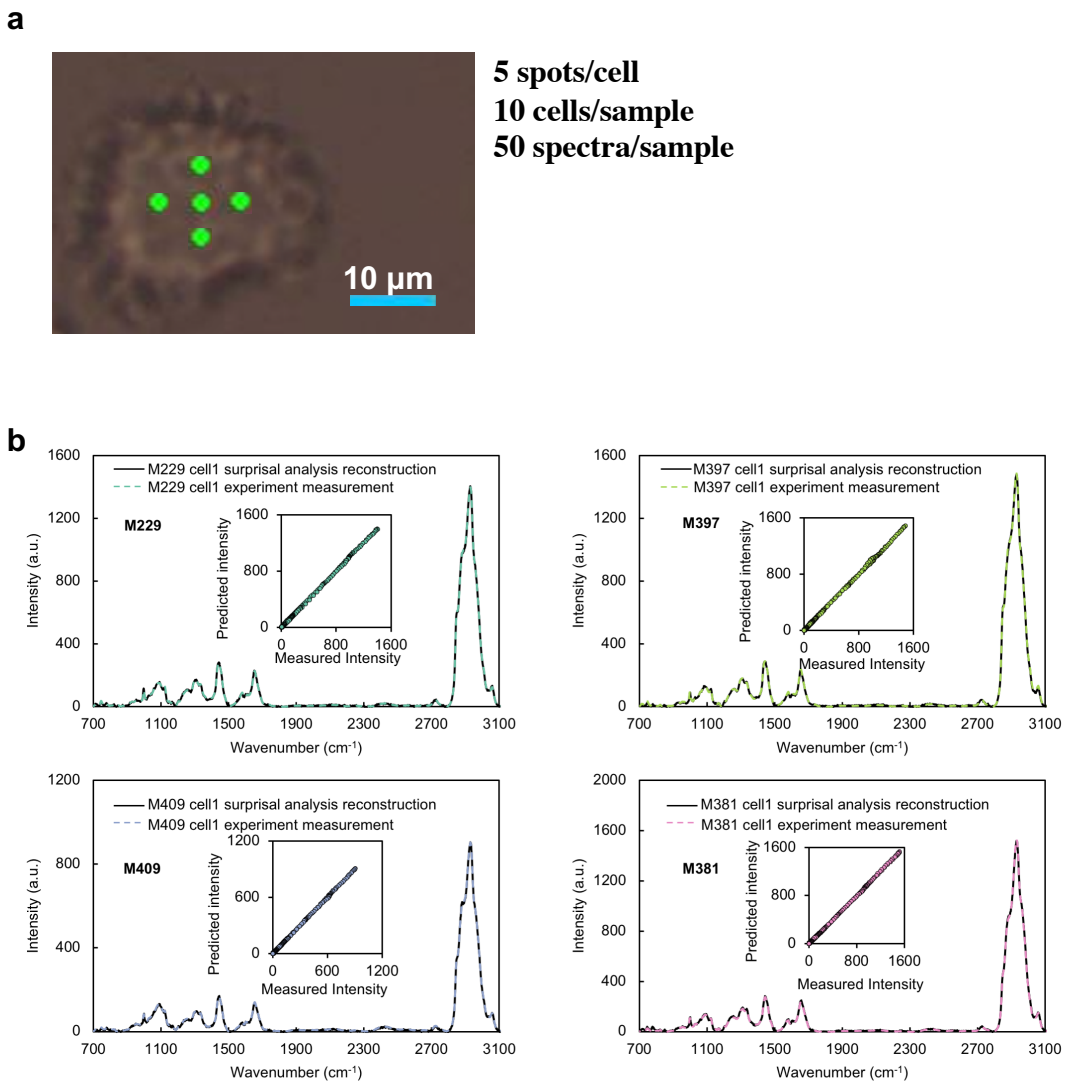
Du *et al*

Supplementary Figures 1 - 13

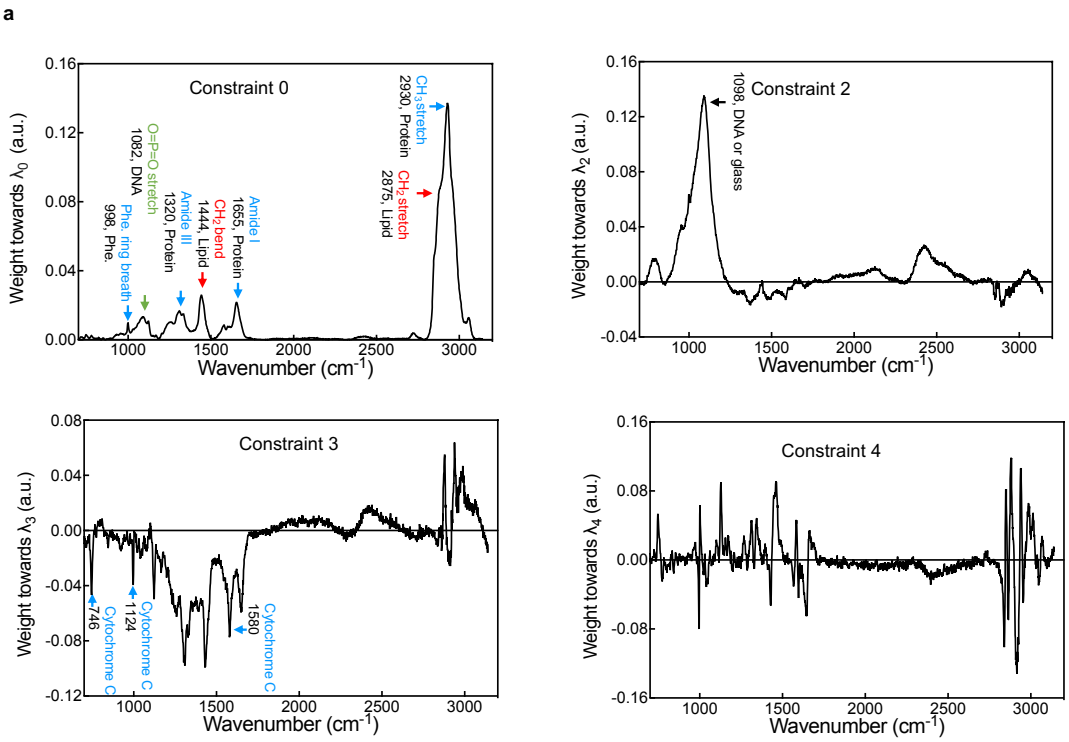
Supplementary Tables 1 – 2



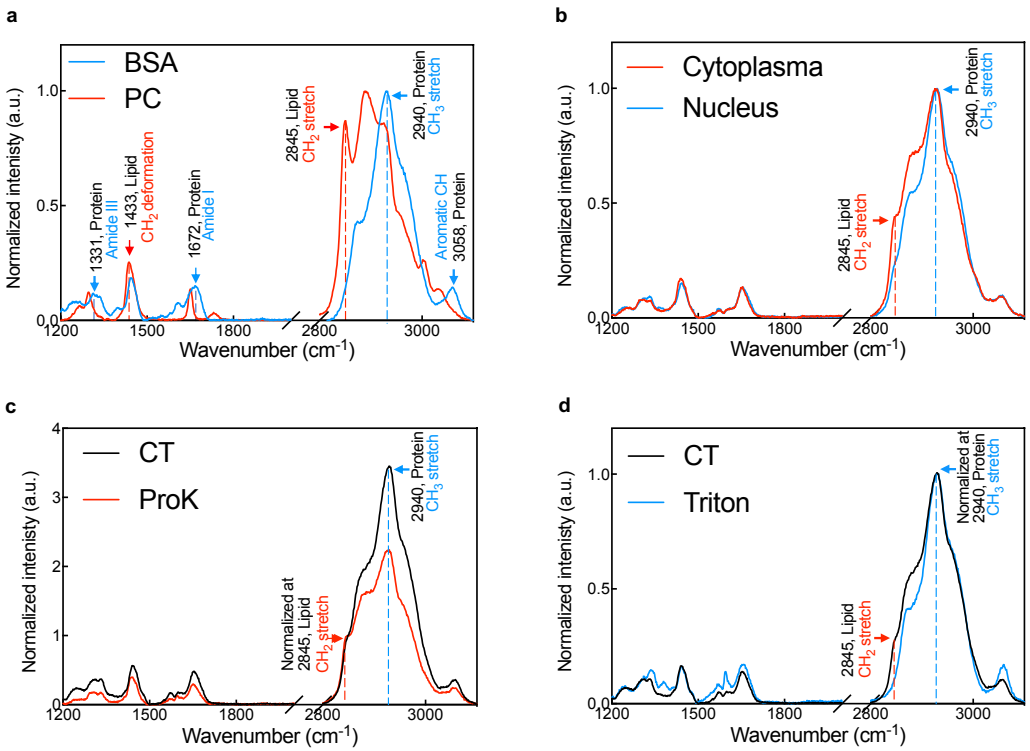
Supplementary Figure 1. Heatmap of the top 100 metabolic genes that are upregulated in each phenotype.



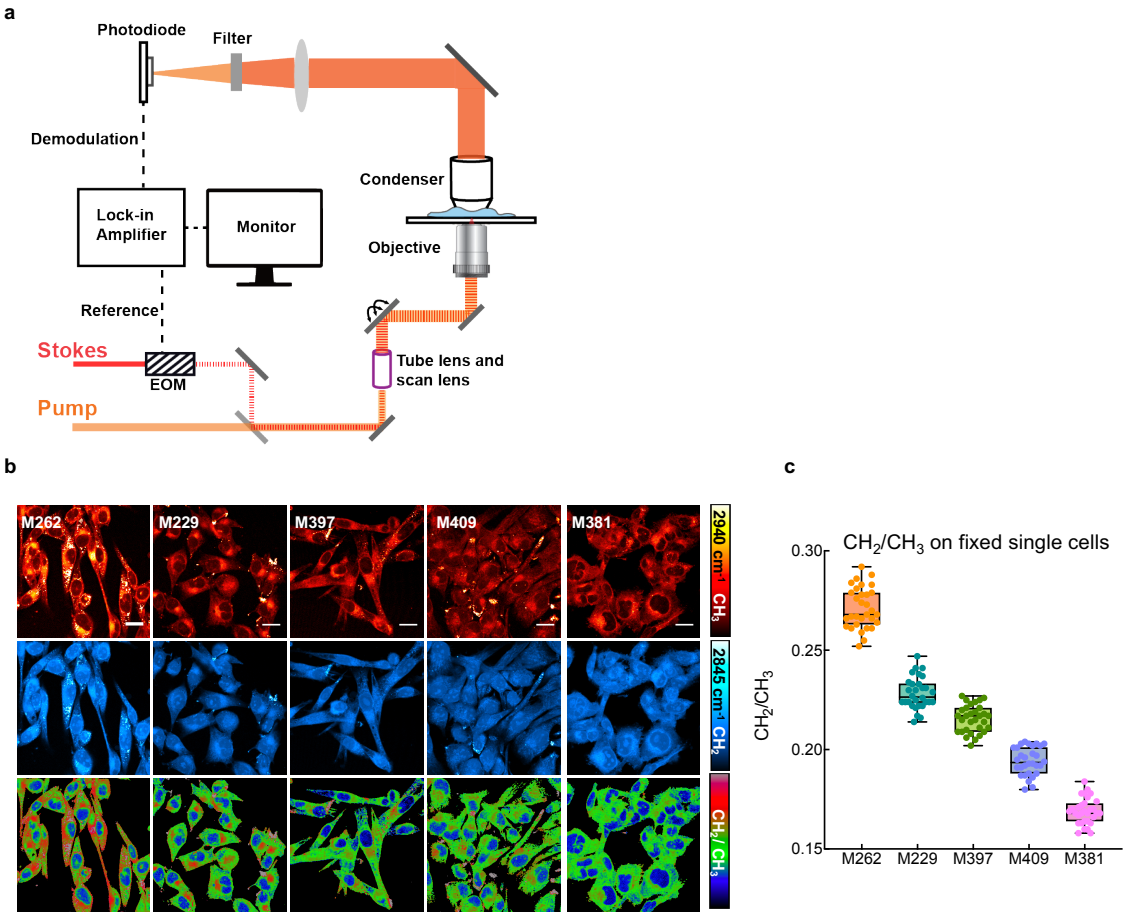
Supplementary Figure 2. Acquisition and surprisal analysis (SA) of spontaneous Raman spectra across the cell lines. a, Illustration of laser focal spots on a representative cell under widefield mode during spontaneous Raman spectra acquisition. Green points indicate the laser focal points on cells. We selected 5 points (center, top, bottom, left, right) on each cell to acquire Raman spectra and averaged them for one cell. We randomly chose 10 cells to represent one cell line. **b,** The comparison between SA reconstructed and experimentally obtained Raman spectra for individual M229, M397, M409, M381 cells. The SA plots are constructed by summing the spectral distribution and amplitudes of the first five resolved constraints (λ_0 - λ_4). The inset plots show the correlation between the predicted and calculated Raman spectra.



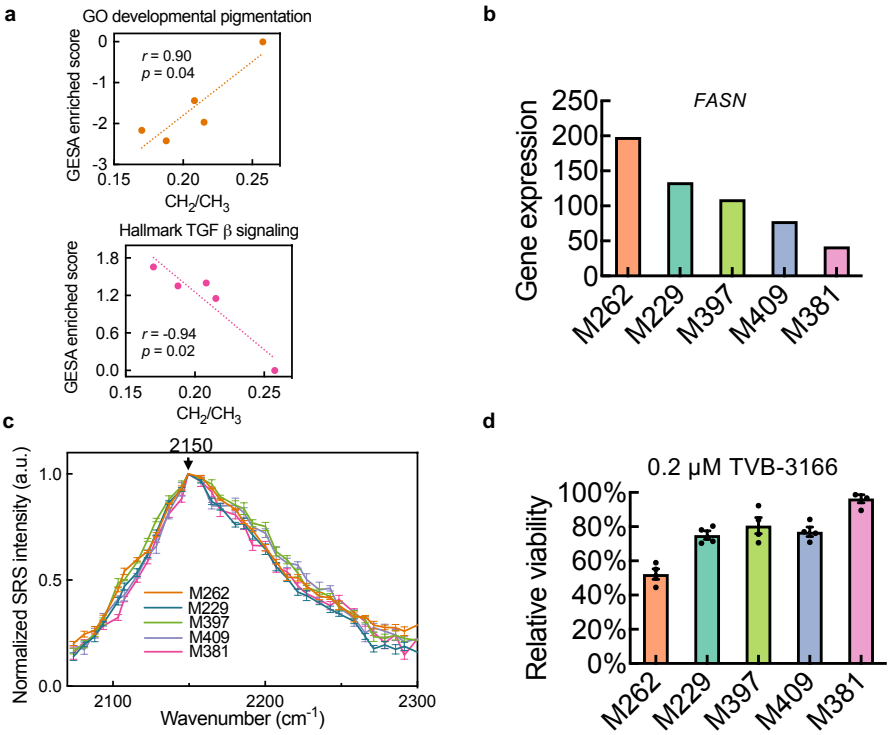
Supplementary Figure 3. Raman peak assignments of the shared constraint (constraint 0, λ_0) and lower amplitude constraints (constraints 2-4, λ_2 - λ_4).



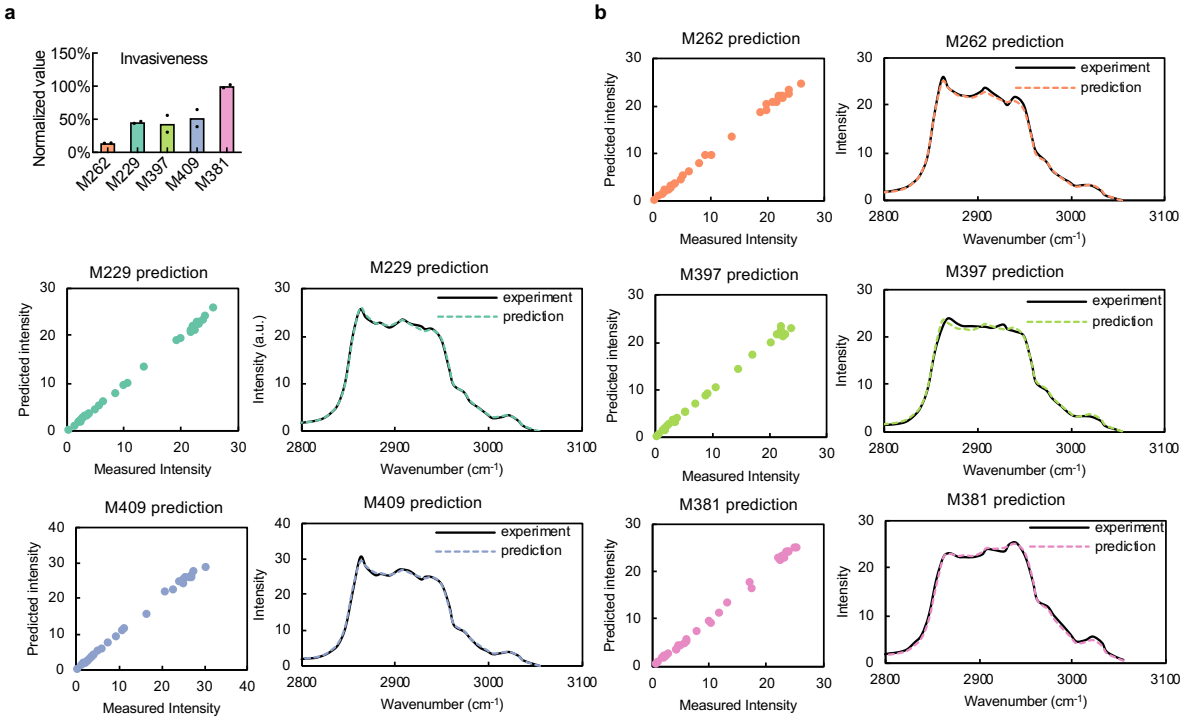
Supplementary Figure 4. Validation of the assignments for the 2845 and 2940 cm^{-1} Raman peaks to lipids and proteins in Fig. 1g. a, Raman spectra of pure proteins (bovine serum albumin, BSA, blue) and lipids (1,2-dioleoyl phosphocholine, the highest abundant lipid in M381 cells from lipidomics, PC, red). 2845 cm^{-1} and 2940 cm^{-1} peaks are dash-line highlighted (the same for all sub-figures). **b,** Raman spectra from cytoplasm (red, more lipid rich) and nucleus (blue, more protein rich) of M381 cells. **c,** Raman spectra of M381 cells before (CT, control, black) and after protease k (ProK, red) treatment to digest most of the proteins. **d,** Raman spectra of M381 cells before (CT, black) and after triton (Triton, red) treatment to wash away most lipids. Spectra in a) and b) are self-normalized, in c) and d) are normalized to 2845 cm^{-1} and 2940 cm^{-1}, respectively.



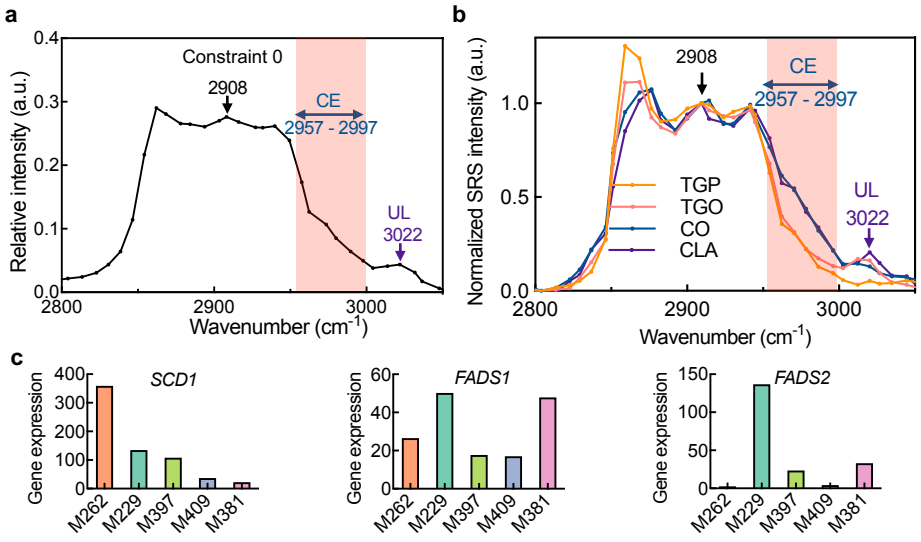
Supplementary Figure 5. SRS imaging of fixed melanoma cells. a, Illustration of SRS configuration. **b,** Representative SRS imaging of fixed melanoma cells at CH_2 (top, 2845 cm^{-1}) and CH_3 (middle, 2940 cm^{-1}) channels. Ratiometric images (bottom, CH_2/CH_3) were generated from the same sets of CH_2 and CH_3 images. Scale bar: 20 μm. **c,** Average single-cell CH_2/CH_3 values from fixed cells for 5 cell lines (n = 30 cells per cell line examined over 3 independent experiments). Data are plotted as boxplots: center line indicates median; box limits indicate upper and lower quartiles; whiskers indicate minimum and maximum. Source data are provided as a Source data file.



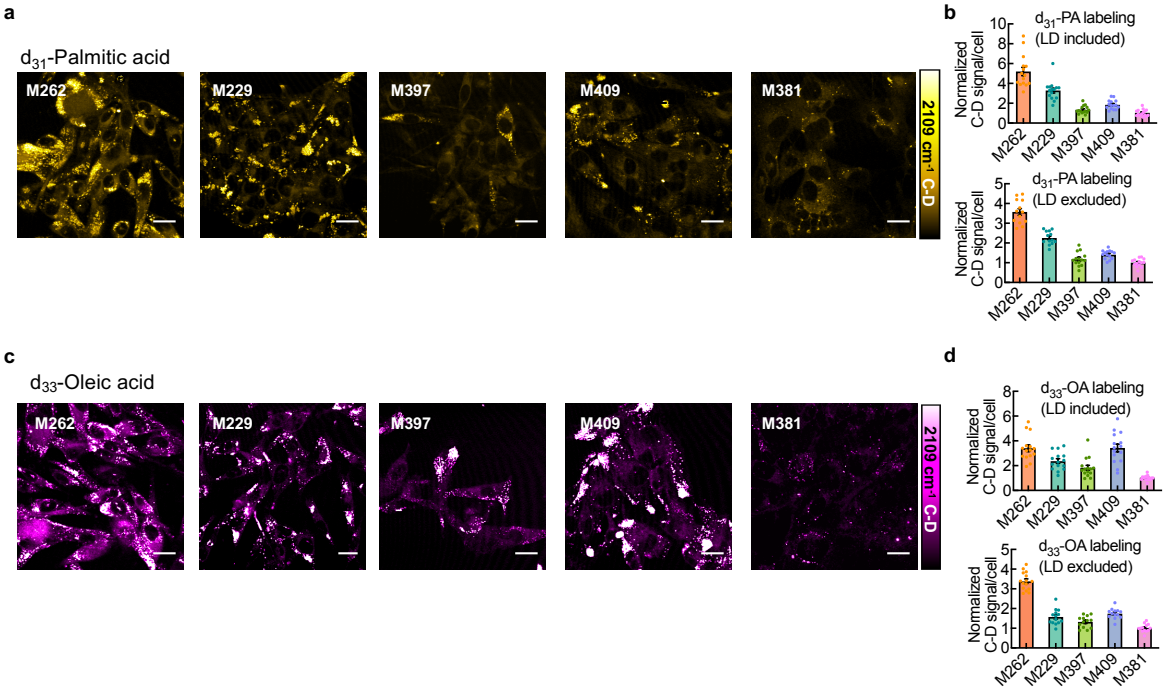
Supplementary Figure 6. The identification of *de novo* fatty acid synthesis as a druggable susceptibility for more differentiated melanoma cells. a, Top panel: cell line-dependent correlation between GSEA scores for the GO developmental pigmentation pathway and CH_2/CH_3 ratios. Bottom panel: cell line-dependent correlation between GSEA scores for TGF beta signaling pathway and CH_2/CH_3 ratios. Each dot represents a two-dimensional relationship for one cell line. **b,** Expression level of FASN across the five different melanoma cell lines (n = 1). **c,** C-D region hyperspectral SRS (hSRS) spectra on cytoplasm in each single d_7-glucose labeled melanoma cells for five cell lines (n = 6, 8, 6, 8, 9 for M262, M229, M397, M409, M381 respectively, data shown as mean ± SEM, spectra are self-normalized). d, Viability tests of melanoma cell lines under 0.2 μM TVB-3166 (FASN inhibitor) treatment for 3 days (n = 4 independent experiments, data shown as mean ± SEM). Source data are provided as a Source data file.



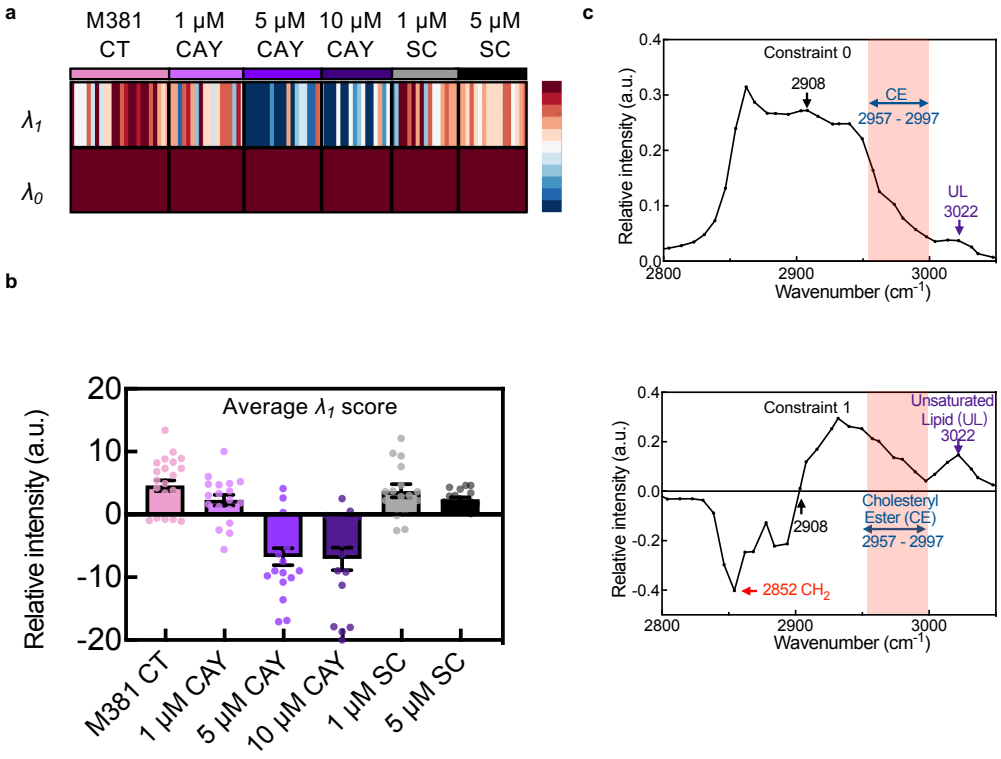
Supplementary Figure 7. Invasiveness assay and validation of SA of hSRS spectra from lipid droplets (LDs). a, Relative invasiveness of different melanoma cell lines quantified by trans-well assay (n = 2 independent experiments). **b,** The comparison between SA reconstructed and experimentally obtained Raman spectra for randomly selected individual lipid droplets from M262, M229, M397, M409, M381 cells. The SA reconstructions utilized only the first two constraints λ_0 and λ_1. Source data are provided as a Source data file.



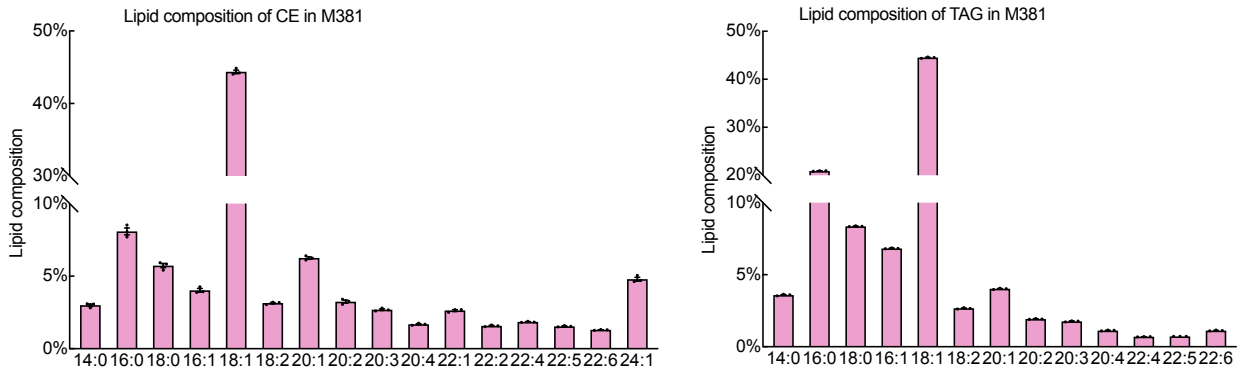
Supplementary Figure 8. Validation of peak assignments in Fig. 3e, and gene expression of fatty acid desaturases across cell lines. a, Peak assignments of constraint 0 (λ_0). The 2908 cm^{-1} reference peak, cholesteryl esters band (CE, 2957 cm^{-1} to 2997 cm^{-1}) and unsaturated lipids peaks (UL, 3022 cm^{-1}) are indicated. **b,** SRS spectra for pure references of glyceryl tripalmitate (TGP), glyceryl trioleate (TGO), cholesteryl oleate (CO), cholesteryl linoleate (CLA) after normalization at 2908 cm^{-1}. The broad band (indicated by pink shadow) ranging from 2957 cm^{-1} to 2997 cm^{-1} features CO and CLA, and is therefore assigned to cholesteryl esters. The 3022 cm^{-1} peak (indicated by violet arrow) is assigned to unsaturated lipid (=C-H), distinct in TGO, CO, CLA. **c,** Gene expression levels of desaturase SCD1, FADS1 and FADS2 across cell lines (n = 1).



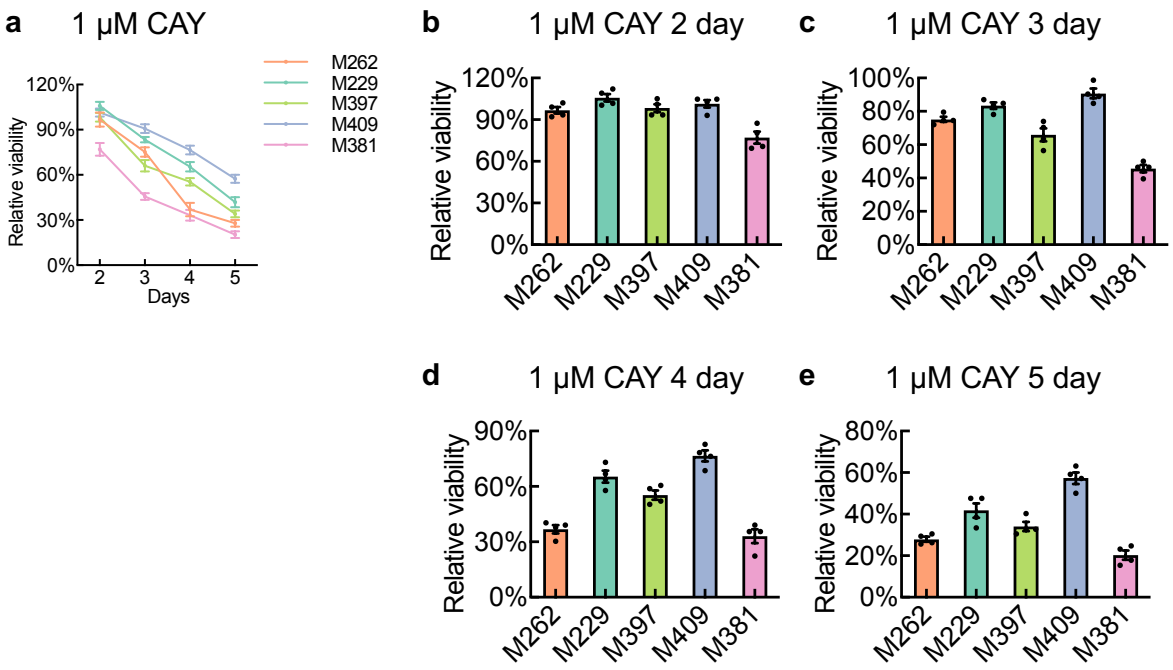
Supplementary Figure 9. Deuterated fatty acids uptake across cell lines. a, Representative C-D channel (at 2109 cm^{-1}) SRS imaging of melanoma cells by incubating cells with d_{31}-palmitic acid (50 μM, 3 days). **b,** Quantification of relative C-D signals in d_{31}-palmitic acid labeling cells at the single-cell level including or excluding LDs (n = 15 cells examined from 3 independent experiments, the C-D signal of M381 cells is normalized to 1). **c,** Representative C-D channel (at 2109 cm^{-1}) SRS imaging of melanoma cells by incubating cells with d_{33}-oleic acid (50 μM, 3 days). **d,** Quantification of relative C-D signals in d_{33}-oleic acid labeling cells at the single-cell level including or excluding LDs (n = 15 cells examined over 3 independent experiments. The C-D signal of M381 cells is normalized to 1. Scale bar, 20 μm. Data shown as mean ± SEM. Source data are provided as a Source data file.



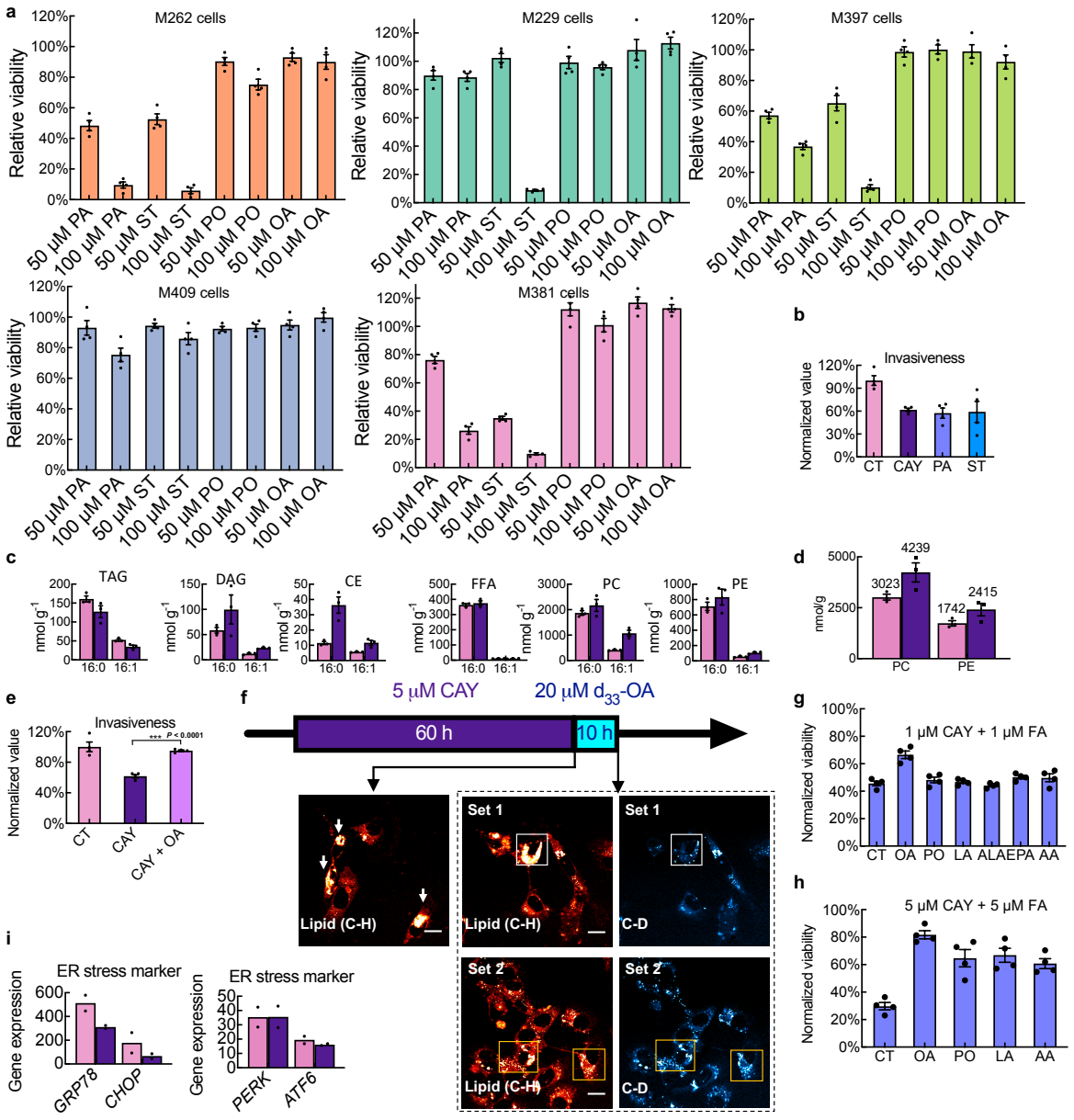
Supplementary Figure 10. SA of hSRS spectra on single LDs in M381 cells with drug treatment. a) Heatmap for scores of the top two constraints (constraint 0 (λ_0) – constraint 1 (λ_1)) by surprisal analysis of hSRS spectra on LDs in M381 cells at different treatment conditions, i.e. M381 control (CT), 1 μM CAY, 5 μM CAY, 10 μM CAY, 1 μM SC, 5 μM SC. Each column represents an individual LD and each row represents the constraint scores. n = 24, 18, 19, 17, 16, 16 for M381 CT, 1 μM CAY, 5 μM CAY, 10 μM CAY, 1 μM SC, 5 μM SC respectively, examined over 3 independent experiments. b) The average score of λ_1 in a) across six treatment conditions. Data shown as mean ± SEM. c) Raman peak assignments for λ_0 and λ_1. The 3022 cm^{-1} peak (violet arrow) is assigned to unsaturated lipids (UL) and the pink shadowed range from 2957 cm^{-1} to 2997 cm^{-1} is assigned to cholesteryl esters (CE). 2908 cm^{-1} is the zero point in λ_1. Source data are provided as a Source data file.



Supplementary Figure 11. Lipidomics of CE and TAG from M381 cells show the involvement of both MUFA and PUFA. Bulk lipidomics of M381 cells with relative percentages of different fatty acid chains in CE (n = 3 independent experiments, left) and in TAG (n = 3 independent experiments, right). Data shown as mean ± SEM. Lipidomics data are provided as Supplementary Data 1. Source data are provided as a Source data file.



Supplementary Figure 12. Viability assays of melanoma cell lines under different days of 1 μM CAY treatment. a, Dependence of viability for cell lines on the treatment length with 1 μM CAY. **b-e,** Bar-chart plot for comparing viability in (a) across five cell lines after 2-day (b), 3-day (c), 4-day (d), and 5-day (e) of CAY treatment. n = 4 independent experiments, Data shown as mean ± SEM. Source data are provided as a Source data file.



Supplementary Figure 13. The mesenchymal M381 is sensitive to saturated fatty acids (SFA) related lipotoxicity. a, Relative viability of cells treated with major SFAs (PA, 16:0 and ST, 18:0), and major MUFAs (PO, 16:1 and OA, 18:1) at indicated concentration for 3 days (n = 4 independent experiments). **b,** Relative invasiveness of control M381 cells (normalized to 100%) and M381 cells after treating with 1 μM CAY, 50 μM PA or 50 μM ST for 3 days. n = 4 independent experiments. **c,** Concentration changes of PA (16:0) and PO (16:1) fatty acid chains in the 6 main species of lipids with (3 days, CAY, purple) and without (CT, pink) CAY treatment from lipidomics (n = 3 independent experiments). **d,** Concentration changes of PC and PE with (3 days, CAY, purple) and without (CT, pink) CAY treatment from lipidomics (n = 3 independent experiments). **e,** Relative invasiveness of control M381 cells (normalized to 100%) and M381 after treating with 1 μM CAY, 1 μM CAY plus 5 μM OA for 3 days (n = 4 independent experiments). **f,** Two color pulse-chase experiments. Top scheme: M381 cells were firstly incubated in medium

containing 5 μM CAY for 60 h (pulse), then the medium was changed to fresh medium containing 20 μM d_{33}-OA but not CAY for 10 h (chase). Representative SRS images at the C-H lipid channel and the C-D channel were shown at two time points of 60 h (1 set, i.e. only pulsed) and 70 h (2 sets, i.e. pulse-chased representative cells are squared). **g,** Relative viability of 1 μM CAY treated M381 cells without (CT) or with rescue of 1 μM indicated unsaturated fatty acid (UFA) for 3 days (n = 4 independent experiments). PO: Palmitoleic acid; LA: Linoleic acid; ALA: Alpha-linoleic acid; EPA: Eicosapentaenoic acid; AA: Arachidonic acid. (n = 4 independent experiments). **h,** Relative viability of 5 μM CAY treated M381 cells without (CT) or with rescue of 5 μM indicated UFA for 3 days (n = 4 independent experiments). **i,** Representative ER stress marker expression with (3 days, purple) and without (pink) CAY treatment (n = 2 independent experiments). Scale bars, 20 μm. Data shown as mean ± SEM. Lipidomics data are provided as Supplementary Data 1. Source data are provided as a Source data file.

Rank	S_1 top 100	S_2 top 100	S_3 top 100	S_4 top 100	Rank	S_1 top 100	S_2 top 100	S_3 top 100	S_4 top 100
1	*TYR*	*RXYLT1*	*ST8SIA5*	*NNMT*	51	*PDE6B*	*GLUD2*	*HS6ST3*	*TYMP*
2	*DCT*	*ALDH1A1*	*GALNT5*	*MGST1*	52	*HSD17B8*	*LPL*	*NPR2*	*RDH10*
3	*GYG2*	*CYP27A1*	*DHRS3*	*GDA*	53	*QDPR*	*CERS1*	*BST1*	*PNMT*
4	*GMPR*	*NPR1*	*ALDH1A3*	*BCAT1*	54	*GCNT3*	*GUCY2C*	*BHMT2*	*AGPAT4*
5	*PNPLA4*	*ATP6V0A4*	*CYB5R2*	*MGAT5B*	55	*PFKFB2*	*PDE7B*	*CSGALNACT1*	*PDE9A*
6	*QPRT*	*GALNT5*	*NT5E*	*HS6ST3*	56	*CYP19A1*	*ASAH1*	*PDE7B*	*ALDOC*
7	*RENBP*	*TYRP1*	*PLA2G7*	*MTAP*	57	*ST8SIA1*	*LDHD*	*GSTM3*	*PDE1C*
8	*ADCY2*	*UROC1*	*CHST1*	*GALNT14*	58	*GSTO2*	*FMO4*	*CYP27C1*	*CYP24A1*
9	*GALNT3*	*FOLH1*	*ACOX2*	*B3GALNT1*	59	*NAT8L*	*CKMT1B*	*PDE9A*	*NME7*
10	*GAPDHS*	*GAL3ST1*	*PDE1C*	*ANPEP*	60	*MGST1*	*EPHX2*	*AK5*	*MFNG*
11	*TYRP1*	*ST8SIA1*	*B3GNT7*	*PTGS2*	61	*RDH8*	*CSGALNACT1*	*AK6*	*DSE*
12	*PRDM7*	*B3GALT1*	*GALNT13*	*CHST15*	62	*GALNT12*	*TPK1*	*INMT*	*TXNRD1*
13	*ADCY1*	*TYR*	*B3GNT5*	*HS3ST3A1*	63	*HSD3B7*	*ALDH1A3*	*DDO*	*B3GNT3*
14	*PIP5K1B*	*DGKI*	*B3GALT2*	*HS3ST3B1*	64	*PIK3C2B*	*BAAT*	*ACSS3*	*PCK1*
15	*ALDOC*	*ENPP2*	*MGLL*	*CYP2S1*	65	*CKMT1A*	*PCYT1B*	*P4HA3*	*CA2*
16	*ATP6V0D2*	*ACP5*	*DPYD*	*PTGES*	66	*INPP4B*	*PDE2A*	*ABHD17C*	*NME3*
17	*BAAT*	*B3GNT7*	*AKR1C3*	*GUCY1A2*	67	*MAOA*	*SCD5*	*DGKA*	*B4GALT1*
18	*LPIN3*	*ALDH2*	*AKR1C1*	*NMNAT2*	68	*LFNG*	*ELOVL2*	*PLCE1*	*PGM2L1*
19	*PLB1*	*DCT*	*ST6GALNAC5*	*LFNG*	69	*ATP8*	*CRYL1*	*SYNJ2*	*CYP26A1*
20	*CA14*	*NT5E*	*TBXAS1*	*DPYD*	70	*CHAC1*	*B3GALT2*	*HSD17B3*	*SYNJ2*
21	*CHSY3*	*ST6GAL1*	*ELOVL4*	*ALPP*	71	*ALDH3B2*	*CKMT1A*	*NMRK1*	*PLCD3*
22	*PTGDS*	*ST8SIA5*	*ABAT*	*CYP1B1*	72	*PLCG2*	*CA12*	*PDE10A*	*ACOT4*
23	*LDHC*	*COLGALT2*	*MGST2*	*CDS1*	73	*NAT8*	*ENPP1*	*GFPT2*	*UGCG*
24	*TKTL1*	*CYP7B1*	*AKR1B10*	*GALNT6*	74	*NAT8B*	*ACOT12*	*COX7A1*	*AMPD3*
25	*OGDHL*	*ASPA*	*UGT8*	*AOX1*	75	*PDE11A*	*CYP19A1*	*ME1*	*GGT5*
26	*PKLR*	*POLR2F*	*PIK3CG*	*ASS1*	76	*GALM*	*PIK3CG*	*GAD1*	*KYNU*
27	*ACP5*	*EXTL1*	*HS3ST5*	*AKR1C3*	77	*ARG2*	*PRDM7*	*ACSM4*	*RRM2*
28	*CA8*	*RENBP*	*PHGDH*	*PLA2G16*	78	*GSTA1*	*CNDP1*	*OPLAH*	*NMNAT3*
29	*UGT2B7*	*XYLT1*	*LPL*	*B3GALT5*	79	*CKB*	*ABAT*	*DSE*	*NQO1*
30	*GLUL*	*ST3GAL6*	*EXTL1*	*ST6GAL2*	80	*PLD1*	*INPP5F*	*PLA2G4C*	*HS6ST2*
31	*PDE3B*	*MAN1C1*	*HEPH*	*GPX3*	81	*HSD17B6*	*GPD1*	*B3GAT1*	*EXT1*
32	*GUCY1A2*	*LARGE1*	*GPX7*	*MAN1A1*	82	*ACOT4*	*IL4I1*	*PIGZ*	*MBOAT7*
33	*CKMT1B*	*LARGE2*	*NMNAT2*	*XDH*	83	*PIP4K2A*	*MGAT5*	*HS3ST3A1*	*PDE6B*
34	*SELENOI*	*GAPDHS*	*CHST2*	*AK5*	84	*ST6GALNAC3*	*FADS2*	*AMT*	*PDE4D*
35	*ACSBG1*	*TBXAS1*	*PCYT1B*	*AK6*	85	*ISYNA1*	*DGKG*	*TUSC3*	*GLUL*
36	*GALC*	*PDE3B*	*ALDH2*	*INPP5J*	86	*ST6GALNAC1*	*HYAL1*	*FADS2*	*NSD1*
37	*PIK3CD*	*PDE3A*	*A4GALT*	*CPT1C*	87	*MAT1A*	*CHST11*	*ENPP4*	*NSD2*
38	*CYP1A1*	*CHST6*	*B4GALNT1*	*ENPP4*	88	*CYP24A1*	*BBOX1*	*UXS1*	*NSD3*
39	*ATP6V0A4*	*GSTM1*	*CHST7*	*CA8*	89	*RDH12*	*ACOX2*	*NOS2*	*ENTPD8*
40	*NPL*	*MAT1A*	*ANPEP*	*ACSL5*	90	*ACER2*	*GDPD1*	*ALDH3B1*	*TCIRG1*
41	*HNMT*	*MGAM*	*AOX1*	*PDE11A*	91	*PDE5A*	*ADCY2*	*XDH*	*CYP27C1*
42	*MGAT4A*	*MGAM2*	*FOLH1*	*HSD17B2*	92	*HYAL1*	*PIGZ*	*MFNG*	*HACD1*
43	*NMRK2*	*GALNT3*	*ETHE1*	*DGKE*	93	*HSD3B2*	*GLDC*	*IDS*	*PLOD2*
44	*B3GALT4*	*CA14*	*RXYLT1*	*PIK3CD*	94	*B3GALNT1*	*DDO*	*PLCH1*	*GCK*
45	*MTAP*	*ENPP3*	*VNN1*	*GBGT1*	95	*ASAH1*	*GNS*	*INPP5F*	*GSTM3*
46	*ST3GAL6*	*CHST7*	*PLOD2*	*GALC*	96	*MTMR8*	*PDXP*	*BCAT1*	*BDH1*
47	*FHIT*	*GSTA4*	*TPH1*	*PDE5A*	97	*LDHD*	*ACSL1*	*PGM3*	*ALDH1L2*
48	*PNLIPRP3*	*UGT2B7*	*CHST3*	*MOCOS*	98	*PDE4D*	*FHIT*	*GCNT1*	*ADK*
49	*HOGA1*	*HS3ST1*	*GALNT18*	*SPTLC3*	99	*HS6ST2*	*HOGA1*	*PTGES*	*IPMK*
50	*DGKE*	*MOGAT1*	*HS3ST1*	*ALPG*	100	*GSTO1*	*PNPLA4*	*A3GALT2*	*MARS*

Supplementary Table 1. The genes list in Supplementary Figure 1. S_1-S_4 columns each indicates the top 100 ranked metabolic genes uniquely upregulated in the melanocytic (S_1), transitory (S_2), neural crest (S_3) and undifferentiated (S_4) phenotypes in Supplementary Figure 1.

Melanoma cell line	Phenotype	IC50 to vemurafenib (nM)	Mutational status
M262	melanocytic	150	BRAFV600E mutant 2 copies BRAF AKT1 mutation&amp CDKN2A deletion
M229	transitory	282	BRAFV600E mutant 4 copies BRAF MITF amplification AKT1 amplification PTEN deletion
M397	transitory	132	BRAFV600E mutant
M409	neural-crest-like	1018	BRAFV600E mutant
M381	mesenchymal	>100000	BRAFV600E mutant

Supplementary Table 2. Basic backgrounds of the five selected melanoma cell lines used in this study.